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14. ABSTRACT To produce a more effective form of trastuzumab and improve efficacy of endostatin, we constructed several anti-HER2 IgG3-endostatin fusion proteins by fusing human endostatin to the 3' end of a humanized anti-HER2 IgG3 antibody. Antibody targeting is designed to enhance local delivery of endostatin to tumor as well as increase endostatin half-life. We constructed two endostatin fusion proteins based on native human endostatin and/or a mutant endostatin with a P125A substitution to confer increased antiangiogenic activity. Native and P125A mutant fusion proteins inhibited tube formation and proliferation of HUVEC in vitro, although P125A fusion protein showed greater inhibition than either native endostatin or endostatin fusion protein. Treatment of established SKBR-3 with the mutant P125A fusion resulted in complete regression of xenografts in SCID mice (5/5 tumor free), compared to untreated, anti-HER2 IgG3, human endostatin, or the native endostatin fusion protein treated mice. Combination of huEndo fusion protein and Avastin synergistically enhanced anti-tumor activity. Linking endostatin to an antibody may significantly enhance anti-tumor activity of trastuzumab. Mutant P125A fusion antibody showed better anti-tumor activity. Targeting antiangiogenic proteins using antibody is a versatile approach that could be applied to other targets (e.g. EGFR, PSMA), or using other antiangiogenic protein domains.					
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BREAST CANCER THERAPY USING ANTIBODY-ENDOSTATIN FUSION PROTEINS

INTRODUCTION

Angiogenesis is critical for growth of primary tumor and for the development of metastasis. Antiangiogenic therapy with agents such as endostatin is under active investigation. Early human trials showed endostatin to be safe, but minimal activity has been observed.¹⁻³ Dosage and schedules may have been suboptimal, and/or late stage disease may not be responsive to recombinant human endostatin. HER2 is overexpressed in 30% of breast cancer and phase II trials of Herceptin demonstrated an 11% response rate in HER2+ patients with metastatic breast disease.⁴⁻⁶ Combining Herceptin with chemotherapy enhances anti-tumor activity resulting in an objective response rate of 60 % or greater in several phase III trials.^{5,7,8} To produce a more effective form of Herceptin and improve the efficacy of endostatin, we have constructed an anti-HER2 IgG3-C_H3-endostatin fusion protein by joining murine endostatin to the 3' end of humanized anti-HER2 IgG3.⁹ Preliminary data using an antibody-murine endostatin fusion protein suggests enhanced effectiveness of anti-HER2 IgG3-endostatin may be due to longer endostatin half-life and the selective targeting of endostatin to tumor by anti-HER2 antibody due to the presence of a fused antiangiogenic factor.⁹

The objective of the proposal is to develop and test novel antibody-fusion proteins with specific ability to deliver antiangiogenic factors to tumors by linking an antiangiogenic factor, human endostatin, with the targeting specificity of an antibody directed against HER2 in order to direct localization of endostatin to the tumor site. Application of the strategy in humans will require careful evaluation of antibody fusion protein antigenicity and might benefit from use of a human endostatin fusion domain. If the antibody-endostatin fusion protein is specifically targeted to the surface of tumor cells, it will be more effective because of retained antibody effector functions, effects on HER2 signaling, and improved ability to inhibit neovascularization in a tumor specific fashion.

To achieve our goals, we set up three specific aims. I. Design and synthesize two variant antibody-human endostatin (huEndo) fusion proteins (anti-HER2 IgG3-Hinge-huEndo and anti-HER2 IgG3-C_H3-huEndo) directed against HER2, which differ in the Fc region and its ability to mediate antibody effector functions (Fig. 1). II. Test the antiangiogenic activity of anti-HER2 antibody-human endostatin fusion protein(s) *in vitro* and *in vivo*. III. Study the antibody-endostatin fusion proteins *in vivo* for effects on tumor growth in animal tumor and/or human xenograft models.



Fig. 1. Schematic diagram of anti-HER2 IgG3-human endostatin fusion proteins. Endostatin domain in orange.

BODY

Specific Aim I. Design and synthesize two variant antibody-human endostatin (huEndo) fusion proteins (anti-HER2 IgG3-Hinge-huEndo (α HER2-H-Endo) and anti-HER2 IgG3-C_H3-huEndo (α HER2-C_H3-Endo)) directed against HER2, which differ in the Fc region and its ability to mediate antibody effector functions.

Task 1. Construction and expression of anti-HER2 IgG3-H-huEndo and anti-HER2 IgG3-C_H3-huEndo fusion proteins (Months 1-6).

Completed in the first year.

Task 2. Produce anti-HER2 H-huEndo and anti-HER2 IgG3-C_H3-huEndo fusion proteins and endostatin (Months 1-24).

Production of endostatin fusion proteins and endostatin

We generated the α HER2-H-Endo and α HER2-C_{H3}-Endo fusion proteins in the first year. We obtained high quantity and good quality of the C_{H3}-Endo fusion proteins (Fig.2, both the native and the mutant type). However, we experienced difficulties with purifying the α HER2-H-Endo using Protein L columns that strongly bind the immunoglobulin light chain, because most of the purified proteins were light chain dimers (50 kDa) instead of the α HER2-H-Endo (170 kDa) fusion protein. We tried to then purify α HER2-H-Endo using either Protein G or Protein A affinity column, but we obtained very low concentrations of H-Endo and the α HER2-H-Endo were co-purified with bovine IgG from culture supernatant with 1% fetal clone sera (serum with very low concentration of bovine IgG). We stopped production of α HER2-H-Endo.

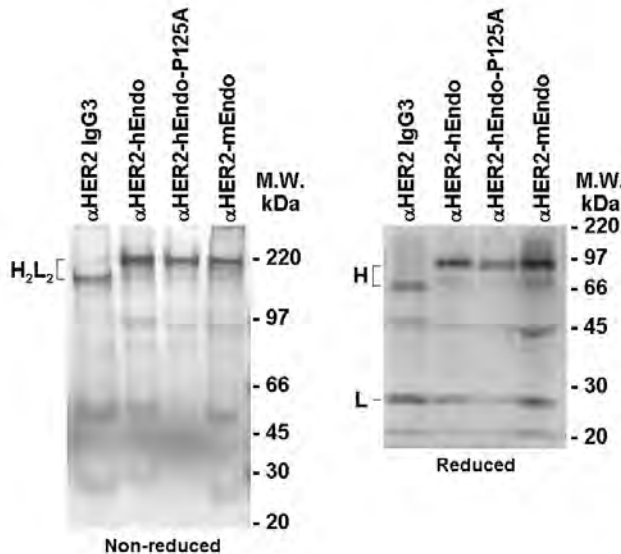


Fig. 2. Expression of anti-HER2 IgG3-C_{H3}-endostatin fusion proteins (the wild type and the mutant type P125A). Secreted human endostatin fusion proteins were labeled with [³⁵S]methionine and immunoprecipitated with Protein A and analyzed under non-reducing and reducing conditions. Control anti-HER2 IgG3-C_{H3}-murine endostatin fusion was used as control.

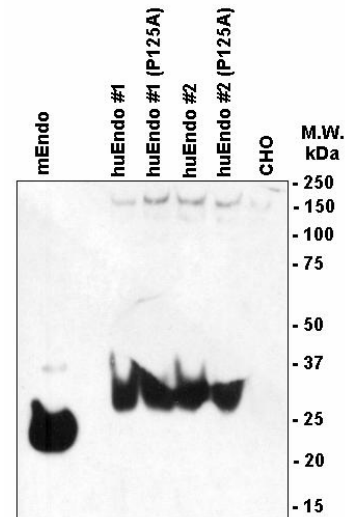


Fig. 3. Expression of human endostatin and a mutant endostatin (P125A). Secreted human endostatin tagged with FLAG was identified with anti-FLAG antibody from the CHO culture supernatant. Murine endostatin tagged with FLAG was used as control.

Human native endostatin (Endo) and a mutant endostatin (A point mutation at position 125 (proline to alanine) has improved endothelial cell binding and antiangiogenic activity.¹⁰⁻¹²) were secreted from CHO cells. Since human endostatin was tagged with FLAG, we detected secreted human endostatin molecules using anti-flag antibody by Western blotting (Fig. 3). Size of human endostatin and human mutant endostatin are bigger than murine endostatin, because human endostatin was tagged with 3x FLAG and murine endostatin tagged with 1x FLAG. We tried to purify endostatin with either anti-FLAG antibody column or heparin sulfate column, but we recovered very little quantities of both human endostatin for our studies. Thus, we decided to do our experiment with commercially available human endostatin, while we continued on solving the problems with purification.

Binding ability to HER2 and antibody-dependent cell-mediated cytotoxicity

To investigate whether the endostatin fusion proteins bind to the HER2 antigen on tumors, frozen section of CT26 and CT26-HER2 tumors were incubated with endostatin fusion proteins and stained with secondary anti-human IgG antibody-HRP, which was used against DAB for visualization (Fig. 4).⁹ The wild type and the mutant type endostatin fusion proteins specifically recognized the HER2 antigen on CT26-HER tumor, but neither of them recognized CT26 tumor.

Since the endostatin fusion domain may affect folding and possibly its antibody effector functions (ADCC or CDC), Anti-HER2 IgG3-C_{H3}-Endo (wild type) was compared with the parental anti-HER2

IgG3 antibody and an irrelevant anti-Dansyl IgG3 antibody (Fig. 5). ADCC activity is determined by a ^{51}Cr -release assay.^{13,14} CT26-HER and SK-BR-3 were used as target cells and CT26 was used as a negative control target. Anti-HER2 IgG3-Endo demonstrated similar ADCC activity of the parental anti-HER2 IgG3 antibody, while the irrelevant antibody showed no ADCC. The endostatin moiety of the fusion protein did not inhibit antibody effector function.

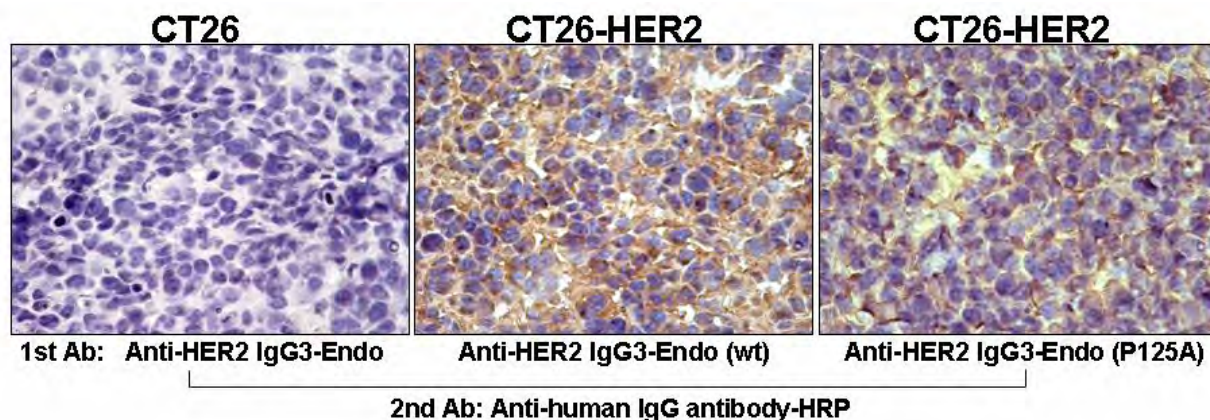


Fig. 4. Targeting of anti-HER2 IgG3-human endostatin fusion proteins to the HER2 antigen on tumors. CT26 and CT26-HER2 tumor sections were incubated with anti-HER2 IgG3-endostatin fusions. Each cryosection was stained with anti-human IgG-HRP and visualized with DAB. All sections were counterstained with hematoxylin.

ADCC with Splenocytes or PBMC

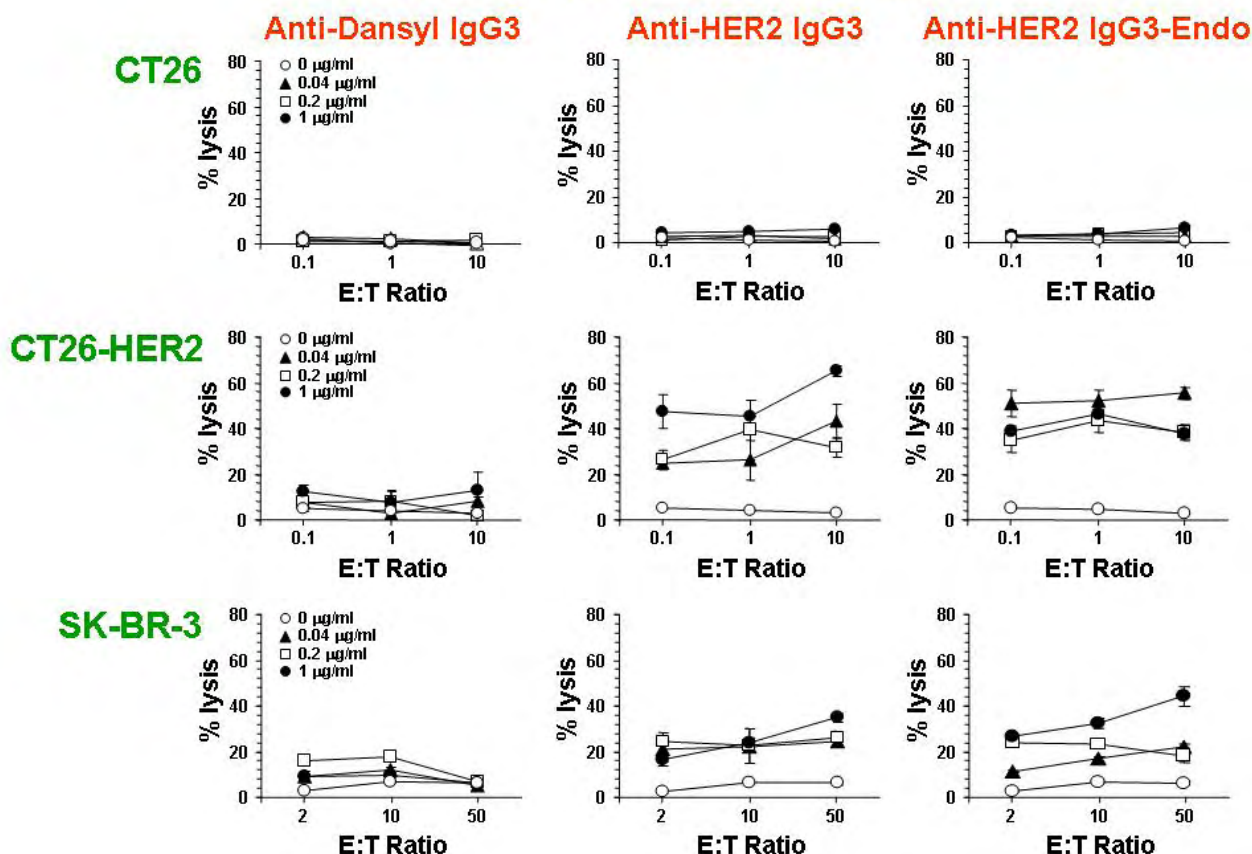


Fig. 5. Antibody-dependent cellular cytotoxicity of CT26-HER2, CT26, or SK-BR-3 cells. ^{51}Cr -labeled target cells were incubated in the presence of various concentrations of antibody fusion proteins plus mouse splenocytes (effector/target ratio: 0.1 to 10) or human PBMC (effector/target ratio: 2 to 50) for 4 hr. Percent specific lysis was calculated as [(experimental release - spontaneous release)/(maximal release - spontaneous release)] \times 100. The data were presented as the means of triplicate determinations \pm SEM.

Task 3. Determine pharmacokinetics, tumor targeting ability, and tissue biolocalization of endostatin fusion proteins (Months 7-12).

Task 4. Analyze antigenicity of the fusion proteins by ELISA (Months 7-12).

Before we determine pharmacokinetics, biolocalization, or antigenicity of endostatin fusion proteins, we would like to examine the antiangiogenic efficacy and anti-tumor ability of the fusion proteins first. If we have any reduced efficacy of the endostatin fusion proteins, we will then revisit these tasks to improve the efficacy of the fusion proteins before moving on.

Specific Aim II. Test the antiangiogenic activity of anti-HER2 antibody-human endostatin fusion protein(s) *in vitro* and *in vivo*.

Task5. Analyze HER2 signaling (Months 4-18).

Anti-proliferation activity of the endostatin fusion proteins on SK-BR-3 tumor cells

Targeting of anti-HER2 IgG-huEndo fusion proteins to tumors may inhibit HER2 signaling. [³H]-thymidine uptake¹⁵ was measured to determine the effect of anti-HER2 antibody-huEndo fusion proteins on proliferation of human breast cancer cell SK-BR-3 (Fig. 6). Endostatin and endostatin fusion proteins inhibited proliferation of SK-BR-3 in a U-shape manner dependent on concentrations.¹⁶ The concentration of 22.73 nM showed the maximal anti-proliferation activity on SK-BR-3 with endostatin or endostatin fusion proteins. SK-BR-3 proliferation was more effectively inhibited by anti-HER2 IgG3-muEndo (α HER2-muEndo, $p = 0.0512$) than murine endostatin (muEndo) and by anti-HER2 IgG3-huEndo (wt, α HER2-huEndo, $p = 0.0053$) and anti-HER2 IgG3-huEndo (mt: P125A, α HER2-huEndo-P125A, $p = 0.0207$) than human endostatin (huEndo). Endostatin fusion proteins were significantly effective than anti-HER2 IgG3 (α HER2 IgG3), but muEndo ($p = 0.0986$) and huEndo ($p = 0.0932$) were not significantly different. In addition, effects on the PI3K/Akt signaling pathways are under examination using Western blot analysis.^{17,18}

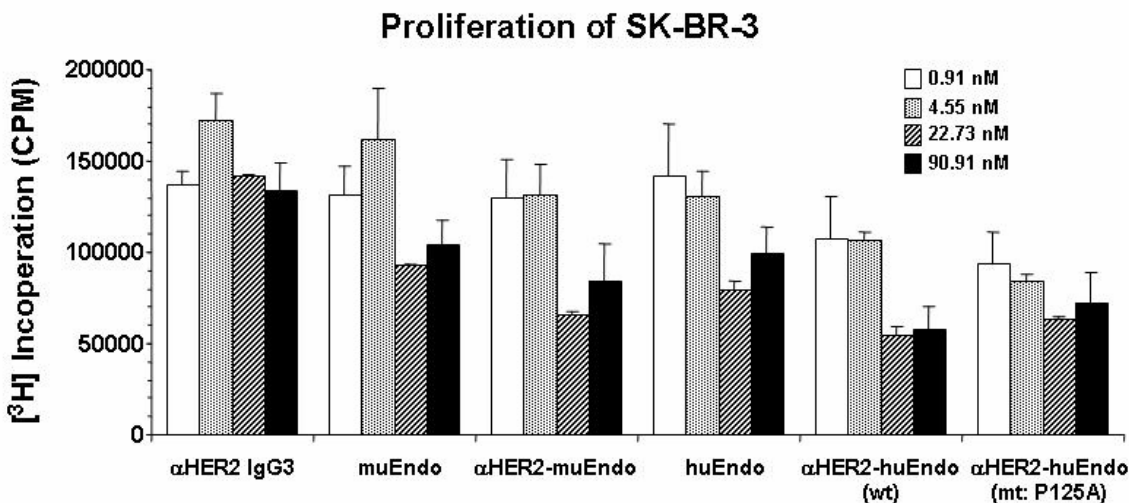


Fig.6. Proliferation of human breast tumor cell, SK-BR-3. SK-BR-3 cells (5000 cells/well) were incubated with endostatin or endostatin fusion proteins at the indicated concentration for 5 days and [³H]-thymidine (1 μ Ci/well) was added 16 hrs before harvesting. The data were presented as the means of triplicate determinations \pm SEM.

Task 6. Analyze antiangiogenic activities (Months 8-18).

Inhibition of endothelial tube formation by the α HER2-huEndo fusion proteins

To evaluate potential functions of the human endostatin moiety in the antibody fusion proteins, we explored the function of the α HER2-huEndo fusion proteins in an *in vitro* morphogenesis assay during which endothelial cells plated on ECM preparations, such as Matrigel, spontaneously aggregate and assemble into densely multicellular capillary-like tubular structures.^{19,20} The α HER2-huEndo fusion proteins treatment at the time of HUVEC plating on Matrigel strongly inhibited assembly into tubular structures, with cells remaining dispersed and exhibiting a morphology resembling cells on plastic rather than aggregation into characteristic capillary-like tubes.²¹ Tubular structures by light microscopy revealed a dose-dependent effect (Fig. 7). The α HER2-huEndo fusion proteins showed significant inhibition of HUVEC tube formation compared to α HER2 IgG3 and human endostatin. In addition, α HER2-huEndo-P125A inhibited more effectively than α HER2-huEndo (Fig. 7). These results suggest that a mutation of proline to alanine at amino acid position 125 of human endostatin may have increased anti-tubule forming efficacy of human endostatin.

Tube Formation of HUVEC

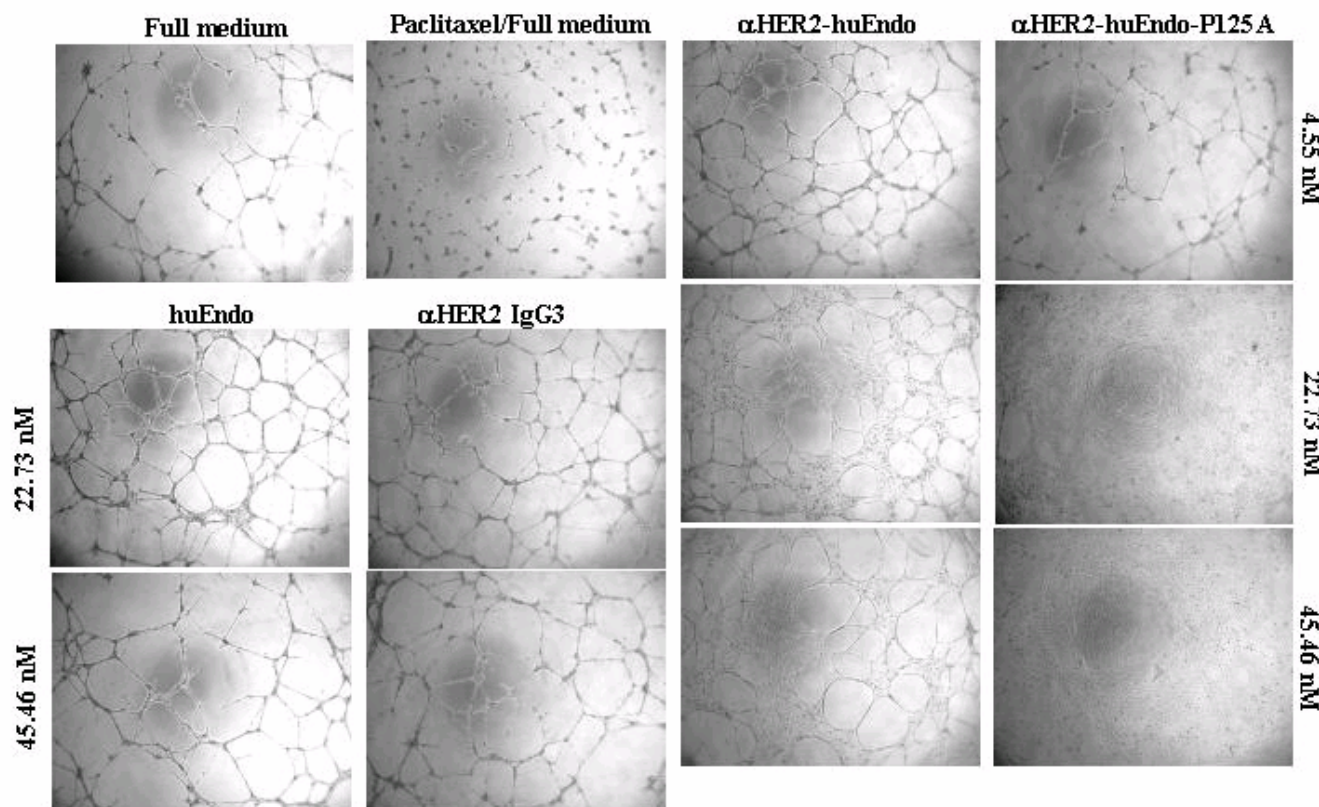


Fig. 7. Effects of anti-HER2 IgG3-huEndo fusion proteins on EC tube formation. HUVECs (4×10^4 cells) were resuspended in 300 μ l of full endothelial cell growth medium and treated with the various anti-HER2 IgG3-huEndo fusion proteins before plating onto the Matrigel-coated plates. After 12-16 hr of incubation, tube formation was observed through an inverted photomicroscope. Full media and paclitaxel were used as negative and positive control, respectively. The data were presented as the means of triplicate determinations \pm SEM.

Proliferation of endothelial cells by the α HER2-huEndo fusion proteins

The effect of the α HER2-huEndo fusion proteins on endothelial cell (EC) proliferation was assessed. HUVECs were exposed to increasing concentrations of the fusion proteins for 72 hrs in the absence or presence of either VEGF or bFGF as angiogenic stimulus.^{19,20} As shown in Fig. 8, both α HER2-huEndo fusion proteins inhibited growth factor-induced proliferation of HUVEC in a concentration-dependent manner. The concentrations necessary for half maximal inhibition (IC50)

were 31.3 nM for α HER2-huEndo and 15.2 nM for α HER2-huEndo-P125A in minimal media (1%FBS/EGM-2), 34.2 nM for α HER2-huEndo and 17.8 nM for α HER2-huEndo-P125A in bFGF/1%FBS/EGM-2, or 33.6 nM for α HER2-huEndo and 15.7 nM for α HER2-huEndo-P125A in VEGF/1%FBS/EGM-2. The α HER2-huEndo fusion proteins were also capable of inhibiting the endothelial cell proliferation stimulated by angiogenic factors, VEGF and bFGF (Fig. 8). HUVEC proliferation was more effectively inhibited by α HER2-huEndo-P125A than by α HER2-huEndo.

Proliferation of HUVEC

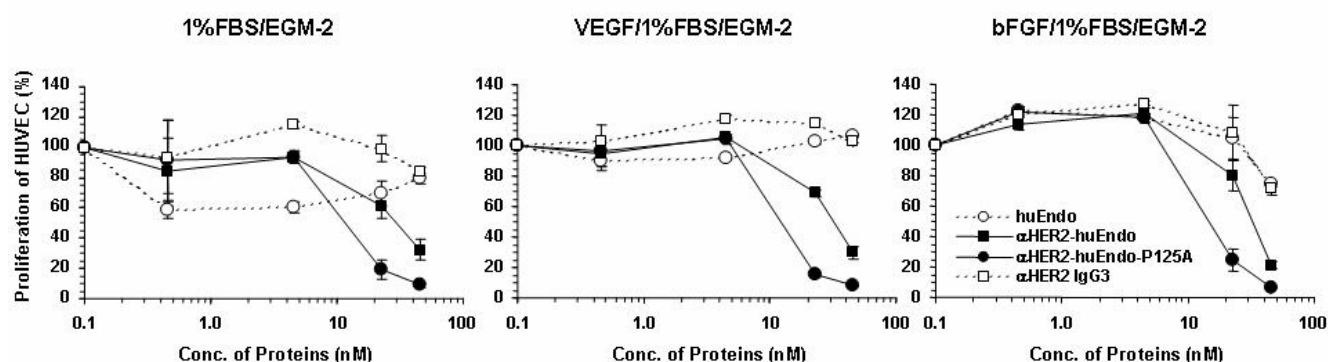


Fig. 8. Effects of anti-HER2 IgG3-huEndo fusion proteins on EC proliferation. HUVECs (4×10^3 cells) were treated with increasing concentrations of the endostatin fusion proteins and proliferation was measured at 72 hrs. The data were presented as the means of triplicate determinations \pm SEM.

Task 7. Analysis of antiangiogenesis (Months 12-24).

Task 8. Examine VEGF/VEGFR and PDGF/PDGFR expression in tumors (Months 12-24).

Once anti-HER2 IgG3-huEndo fusion proteins demonstrated anti-proliferation of EC, we investigated anti-tumor activities, before we examined the mechanism of anti-proliferation of EC. If we observed enhanced anti-tumor activity, we would revisit task 7 and 8, and further investigate the anti-tumor mechanism. In fact, we are now in the process of examining these tasks, as subsequent studies showed anti-HER2 IgG3-huEndo fusion proteins demonstrated enhanced anti-tumor efficacy (please see below).

Specific Aim III. Study the antibody-endostatin fusion proteins *in vivo* for effects on tumor growth in animal tumor and/or human xenograft models.

Task 9. Anti-tumor activity in human tumor xenografts (Months 18-24)

Anti-tumor efficacy in human breast cancer SK-BR-3 xenografts

Herceptin, anti-HER2 IgG1, is able to inhibit the growth of human breast cancer SK-BR-3 overexpressing HER2. SK-BR-3 may need both signaling through HER2 and neoangiogenesis for optimal growth. We assayed for anti-tumor activity of α HER2-huEndo fusion proteins against human breast cancer SK-BR-3 in SCID mice.⁹ Equimolar proteins were injected every other day. The SK-BR-3 tumor xenografts showed the greatest differences among the treatments (Fig. 9). In Fig. 9A, huEndo and α HER2 IgG3 inhibited 48.36% (p value = 0.0123) and 55.46% (p value = 0.0046) of tumor growth relative to the non-treated group (PBS) on day 29, while α HER2-huEndo and α HER2-huEndo-P125A inhibited 82.58% (p value = 0.0111) and 98.66% (p value = 0.0090), respectively. It is noteworthy that the treatment with α HER2-huEndo-P125A completely eradicated tumors after 30 days. The proportion

of tumor-free survivors was higher for the α HER2-huEndo-P125A group (5 of 5) compared to PBS (0 of 5), α HER2 IgG3 and huEndo (1 of 5), and α HER2-huEndo (2 of 5) (Fig.9B). α HER2-huEndo-P125A showed a greater extent survival rate of the human breast cancer SK-BR-3 xenografts in SCID mice than α HER2 IgG3, huEndo, or α HER2-huEndo.

A. Tumor growth of SCID mice bearing SKBR-3 tumor

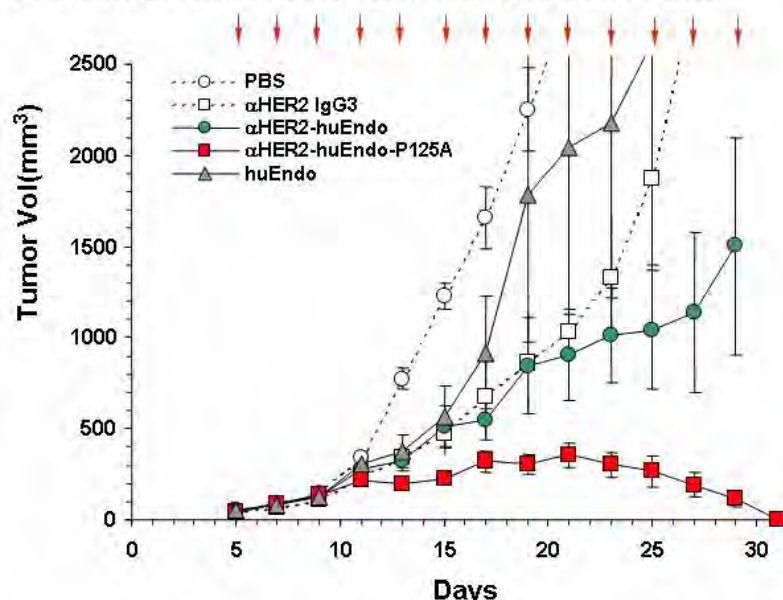
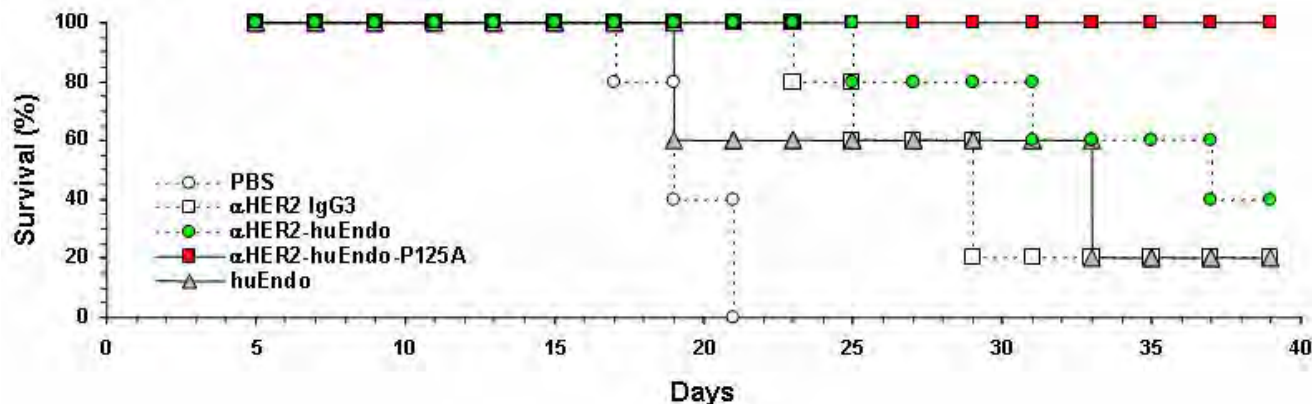


Fig. 9. Anti-tumor efficacy of anti-HER2 IgG3-huEndo fusion proteins. SCID mice (n=5) were *s.c.* inoculated with 2×10^6 SK-BR-3 on the right flank back on day 0, then the mice were *i.v.* injected with anti-HER2 IgG3-huEndo (42 μ g), antiHER2 IgG3 (34.9 μ g), human endostatin (8 μ g), or PBS every other day (indicated with arrow). (A) Tumor growth was monitored every day, and the size of tumor was measured with calipers. Tumor volume was calculated as $4/3 \times 3.14 \times \{(\text{long axis} + \text{short axis})/4\}^3$. The values represent mean \pm SEM of tumor volume (mm^3) of 5 mice. (B) Survival of mice per treatment group. Mouse with more than 2000 mm^3 in volume was euthanized.

B. Survival of SCID mice bearing SKBR-3 tumor

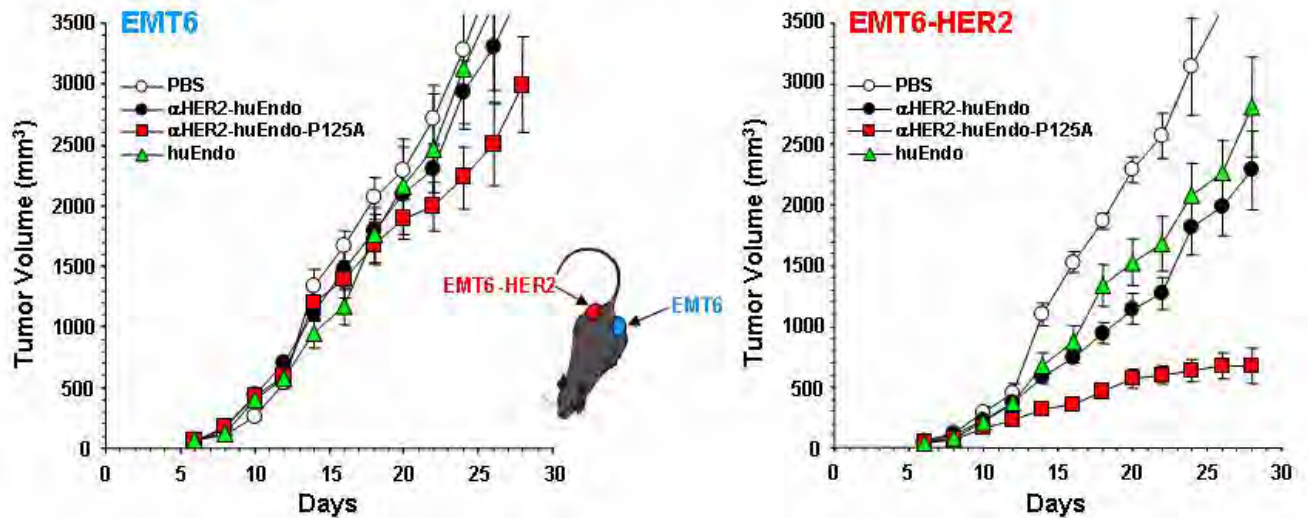


Anti-tumor efficacy in murine mammary tumor EMT6 model

In previous study, we demonstrated that the *in vivo* targeting of antiangiogenic proteins using α HER2-murine Endo fusion protein enhanced preferential inhibition of tumor growth expressing HER2, compared to contralaterally implanted parental tumor (no HER2 expression). To investigate whether the ability of α HER2-huEndo fusion proteins to specifically target tumors bearing HER2 may increase efficacy, BALB/c mice simultaneously implanted with mouse mammary EMT6 and EMT6-HER2 tumors on opposite flanks were treated with either α HER2-huEndo-P125A, α HER2-huEndo, or huEndo.⁹ Equimolar administration of α HER2-huEndo-P125A to mice showed preferential growth inhibition of EMT6-HER2, compared to EMT6 parental tumor implanted on the contralateral flank (Fig. 10). The ability of α HER2-huEndo fusion proteins to specifically target tumors bearing HER2 increased anti-tumor efficacy. α HER2-huEndo-P125A inhibited EMT6-HER2 tumor growth more effectively than PBS (p value = 0.0025), huEndo (p value = 0.0031), or α HER2-huEndo (p value =

0.0041). The mutation of human endostatin moiety of α HER2-huEndo-P125A fusion protein therefore increased the potential efficacy of current antiangiogenic strategies of α HER2-huEndo fusion proteins.

A. Tumor growth of BALB/c mice bearing EMT6 and EMT6-HER2



B. Individual tumor growth of BALB/c mice bearing EMT6 and EMT6-HER2

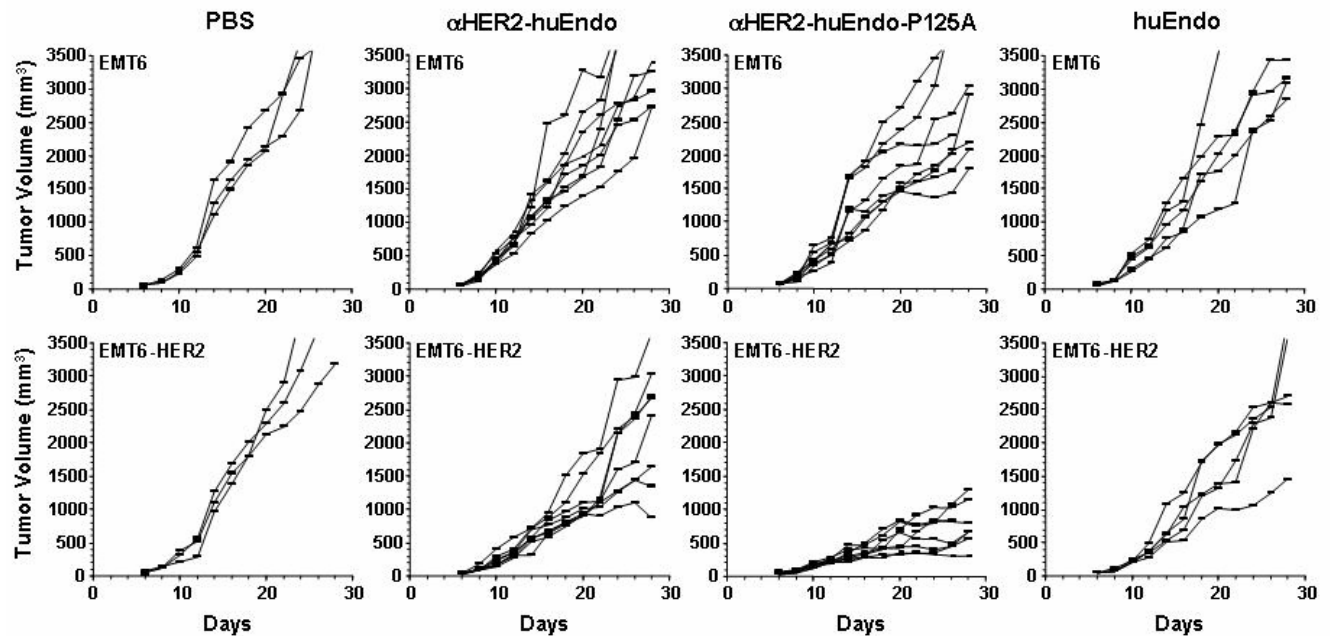


Fig. 10. Anti-tumor activity of α HER2-huEndo fusion proteins in a syngeneic mouse model. A. Tumor growth of mice bearing murine mammary tumor EMT6 and EMT6-HER2. BALB/c mice ($n=3-8$ per group) were implanted *s.c.* contralaterally with EMT6 and EMT6-HER2 (1×10^6 cells per mouse), followed on day 6 by equimolar injections every other day (11 times) of anti-HER2 IgG3-huEndo (42 μ g), human endostatin (8 μ g), or PBS. Tumor measurements are described above and are presented as mean \pm SEM. B. Individual tumor growth of mice treated with anti-HER2-huEndo fusion proteins.

Task 10. Anti-tumor activity in metastatic models (Months 21-27)

Task 11 (old Task 12: Mislabeled). Anti-tumor activity in orthotopic metastatic models (Months 24-36)

In the third year, we will continue to focus on testing efficacy of the fusion proteins *in vivo* in other tumor models. We have already developed another murine mammary tumor model, 4T1 and 4T1-HER2, which is well known as a murine orthotopic breast metastatic tumor.

Task 12 (old Task 13: Mislabeled). Combination treatment (Months 24-36)

PDGF blockade: Imatinib (Months 24-30)

VEGF blockade: Avastin (Months 24-30)

Metronomic therapy (Months 27-36)

We started the combination therapy with anti-HER2 IgG3-huEndo (wild type) protein only, because anti-HER2 IgG3-huEndo-P125A (mutant type) alone was able to show great enhanced anti-tumor activity. In the third year, we will continue to investigate synergistic anti-tumor activity with the combination therapy.

PDGF blockade: Imatinib (Gleevec)

Gleevec (STI57, imatinib, Novartis Pharma AG) has been approved for chronic myelogenous leukemia and gastrointestinal stromal tumors. Gleevec disrupts the association of pericytes with neovasculature in tumors through its effects on PDGFR.²² While endostatin inhibits early blood vessel formation, imatinib may affect maturation by acting on pericytes.^{22,23} We initially treated EMT6 and EMT6-HER2 tumors, subcutaneously implanted on the left and right flank respectively, with combination of anti-HER2-huEndo fusion protein and gleevec.⁹ However, EMT6 and EMT6-HER2 tumors were very sensitive to gleevec, and no additive or synergistic effect was observed. We repeated the gleevec combination therapy on the CT26 and CT26-HER2 tumor model (Fig. 11). Although CT26/CT26-HER2 tumors were less sensitive to gleevec than EMT6/EMT6-HER2, the combination of α HER2-huEndo and gleevec did not show significantly increased anti-tumor efficacy compared to α HER2-huEndo ($p = 0.118$) or gleevec alone ($p = 0.070$). We will attempt to examine whether SK-BR-3, 4T1/4T1-HER2, or MCF7/MCF7-HER2 tumor cell lines are sensitive to gleevec in the third year.

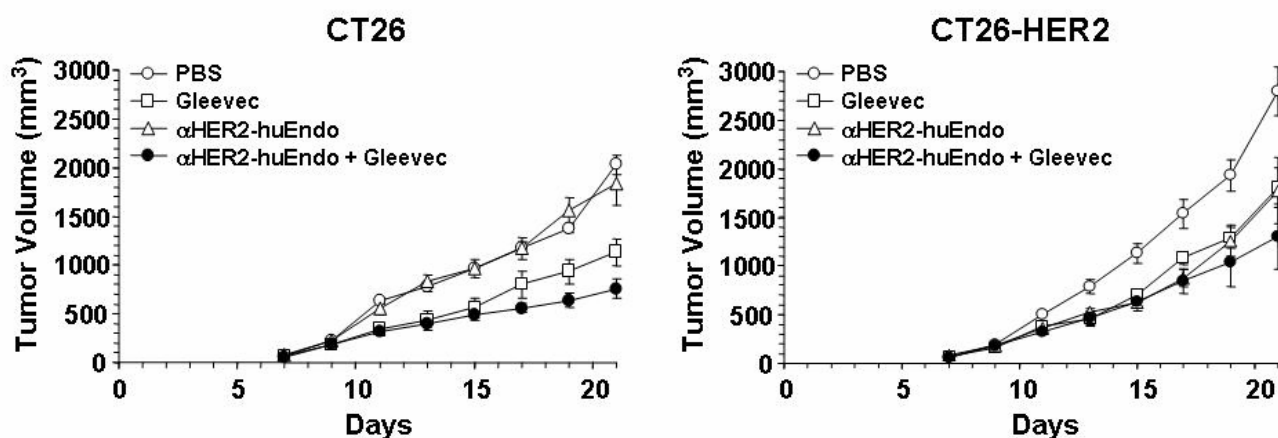


Fig. 11. Evaluation of the combination therapeutic effect with gleevec on established tumor. BALB/c mice (n=5) were implanted *s.c.* contralaterally with CT26 and CT26-HER2 (1×10^6 cells per mouse), followed on day 7 by every other day of α HER2-huEndo (42 μ g), gleevec (*p.o.* 2 mg/day), or combination of α HER2-huEndo and gleevec. Tumor measurements are described above and are presented as mean \pm SEM.

VEGF Blockade: Bevacizumab (Avastin)

A humanized anti-VEGF antibody (bevacizumab, AvastinTM, rhuMAb-VEGF; Genentech) binds and neutralizes all of the major isoforms of VEGF-A, decreasing vascular volume, microvascular

density, interstitial fluid pressure and the number of viable, circulating endothelial cells.²⁴ Therefore, combining fusion proteins with Avastin may augment activity of both approaches. We studied whether α HER2-huEndo, Avastin, α HER2 IgG3, or both α HER2-huEndo and Avastin in combination would inhibit the growth of human breast cancer SK-BR-3 xenografts in SCID mice. SK-BR-3 was implanted on the flank of SCID mice.⁹ The treatment was repeated (shown below in Fig. 12). Administration of α HER2-huEndo or Avastin alone showed enhanced anti-tumor activity, compared to either α HER2 IgG3 alone ($p=0.0208$, 0.0387 respectively), or the untreated group ($p=0.0135$, 0.0150 respectively). As expected, α HER2-huEndo and Avastin given in combination resulted in a significantly and synergistically greater reduction of tumor volume, compared to either α HER2-huEndo ($p=0.0051$), Avastin ($p=0.0036$), α HER2 IgG3 ($p=0.0149$), or the untreated group ($p=0.0141$) (Fig. 12).

A. Tumor growth of SCID bearing SK-BR-3

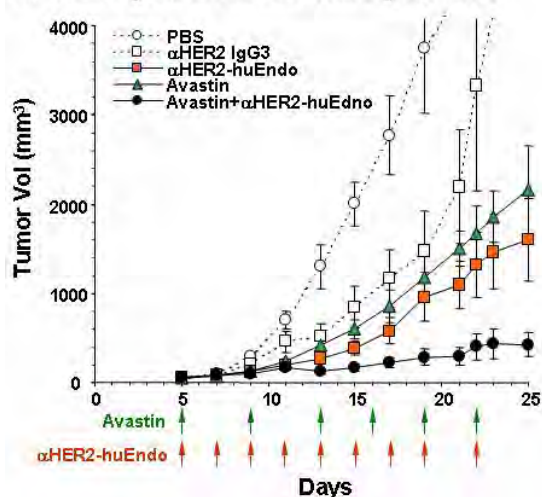
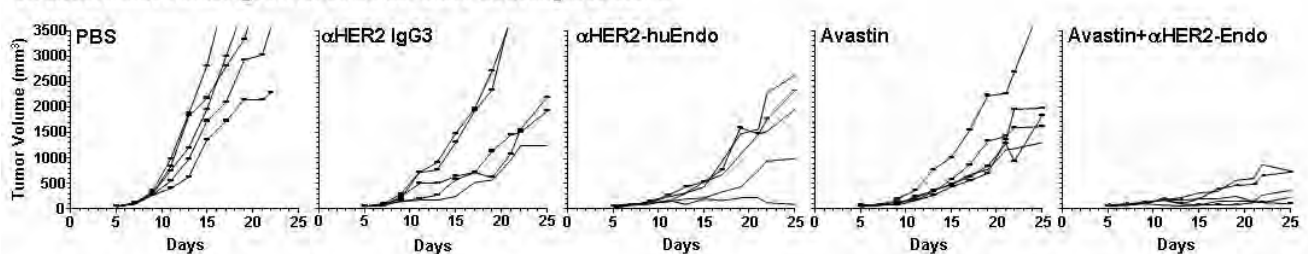


Fig. 12. Evaluation of the combination therapeutic effect with Avastin on established tumor. A. Tumor growth of mice bearing human breast tumor SK-BR-3. SCID mice ($n=5$) were implanted *s.c.* with SK-BR-3 (2×10^6 cells per mouse), followed on day 5 by every other day of α HER2-huEndo (*i.v.* 42 μ g, every other day), Avastin (*i.p.* 200 μ g, twice a week), or combination of α HER2-huEndo and Avastin. Tumor measurements are described above and are presented as mean \pm SEM. B. Individual tumor growth of mice treated with α HER2-huEndo, Avastin, or combination of α HER2-huEndo and Avastin.

B. Individual tumor growth of SCID mice bearing SK-BR-3



KEY RESEARCH ACCOMPLISHMENTS

1. Targeting of α HER2-huEndo fusion proteins to tumor is important the inhibition of tumor growth.
2. Both α HER2-huEndo fusion proteins enhanced anti-tumor efficacy, but α HER2-huEndo-P125A (mt) fusion has a significantly augmented inhibition of tumor growth.
3. Combination of α HER2-huEndo and Avastin synergistically enhanced anti-tumor activity.

REPORTABLE OUTCOMES

We are preparing a patent application with α HER2-huEndo-P125A (mt) fusion and a manuscript.

CONCLUSIONS

Linking human endostatin to an antibody may significantly enhance anti-tumor activity of trastuzumab. Mutant P125A fusion antibody showed much better anti-tumor activity. Combination with other antiangiogenic drugs synergistically augmented anti-tumor efficacy. Targeting

antiangiogenic proteins using antibody is a versatile approach that could be applied to other targets (*e.g.* EGFR, PSMA), or using other antiangiogenic protein domains.

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APPENDICES

Not applicable.

SUPPORTING DATA

Not applicable.